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ORIGINAL RESEARCH

Genome Targeted Introgression of Resistance to African Stem Rust from *Aegilops sharonensis* into Bread Wheat

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Abstract

Many accessions of the wheat wild relative Sharon goatgrass (*Aegilops sharonensis* Eig., *AES*) are resistant to African races of the stem rust pathogen (i.e., Ug99 group races), which currently threaten wheat production worldwide. A procedure was designed to introgress the respective resistances to specific bread wheat genomes by producing plants homozygous for the A and B genomes and hemizygous for the D and S^{sh} genomes or homozygous for the A and D genomes and hemizygous for the B and S^{sh} genomes. In these genotypes, which lack the *Ph1* allele, homeologous pairing was expected mainly between chromosomes of the D and S^{sh} genomes or B and S^{sh} genomes, respectively. An antigametocidal (AG) wheat mutant (*Gc2*^{mut}/*Gc2*^{mut}) was used to overcome gametocidal effects. Wheat lines initially found resistant at the seedling stage were also highly resistant at the adult plant stage in rust nurseries established in the field. DNA of 41 selected homozygous resistant lines, analyzed by the Axiom wheat 820K SNP array, showed alien chromatin mainly in wheat chromosomes 1B, 1D, and 5B. This work suggests that, in most cases, it is possible to target introgressions into the homeologous chromosome of a selected genome of bread wheat.

Core Ideas

- A method was designed to target alien homoeologous introgression into a selected genome
- Stem rust resistant wheat lines were produced accordingly
- Axiom array DNA analysis confirmed the introgression into the targeted genome

WIDELY virulent races of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*, *Pgt*) from Africa (i.e., the "Ug99 race group") threaten wheat production in many regions around the world. Host resistance is the preferred method for combating the disease; however, only a limited number of resistance sources effective against the Ug99 race group has been identified within the readily accessible primary gene pool of wheat (Singh et al., 2015). Members of the secondary genepool of wheat, particularly those in the Sitopsis section of *Aegilops* (comprising diploid species with S or modified S genome), are rich sources of resistance to the Ug99 race group. For example, in seedling evaluations against race TTKSK, the

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Abbreviations: *AES*, *Aegilops sharonensis* Eig; AG, antigametocidal; BC, backcross(ed); BSL, biosafety level; CNV, copy number variation; CS, 'Chinese Spring'; IT, infection type; SP, self-pollinate(d).

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original type race of the Ug99 race group, 69 to 77% of *AES* accessions were resistant (Olivera et al., 2007; Scott et al., 2014). Genetic studies on selected *AES* accessions using North American *Pgt* races revealed that resistance was controlled by one or two dominant genes (Olivera et al., 2008). Recently, Yu et al. (2017) mapped two resistance genes effective against the Ug99 race group on chromosomes $1S^{sh}$ and $5S^{sh}$ of the *AES* accession AEG-1644.

The genetic diversity of bread wheat for resistance to the Ug99 race group can be greatly enhanced with genes introgressed from *AES*. Bread wheat is an allohexaploid organism composed of A, B, and D genomes but inherently allows only intragenomic (homologous) pairing and prevents intergenomic (homeologous) pairing. Similarly, chromosomes of additional genomes such as S^{sh} of *AES* will not regularly pair with their wheat homeologues—a situation that prevents recombination, which is required for alien gene transfer. Some methods were developed to surmount this difficulty. The basic process for gene introgression is routine, but cumbersome and time-consuming to complete (Millet et al., 2014). Moreover, two key obstacles have to be overcome, namely induction of homeologous recombination and elimination of the gametocidal effect. In this investigation, we modified a chromosome engineering technology such that genes for resistance to race TTKSK from *AES* accessions AEG-1644 and AEG-2172 were successfully transferred into bread wheat. Since this manipulation substitutes an alien segment for its wheat homeologue, it is desirable to preferentially induce recombination with a specific wheat homeologous chromosome (i.e., an *AES* introgression from $1S^{sh}$ into 1B as opposed to 1D). The method described herein was designed to induce recombination with either the B or D genome of wheat.

Another important issue which had to be solved is how to identify the location and size of the alien introgressions. Recently, King et al. (2017) pioneered a detection method for a large number of introgressions from *Amblyopyrum muticum* into wheat using a subset of validated SNPs from the ultra-high-density Axiom genotyping array that was designed and used for genotyping wheat and its relatives (Winfield et al., 2016). This high density array was used here for the first time to identify alien chromatin relating to a selected trait.

Materials and Methods

Plant Material

Two resistant *AES* accessions were obtained from the Harold and Adele Lieberman Germplasm Bank at the Institute for Cereal Crops Improvement, Tel Aviv University and used in this investigation: AEG-1644 collected in Ashdod, Israel, and AEG-2172 collected in Qiryat Ono, Israel. These two genotypes exhibited a high level of resistance (low infection types [ITs] of 0;) to race TTKSK (Olivera et al., 2007, Supplementary Table S1).

Two wheat mutants were used to induce homeologous pairing in different cross combinations: bread wheat 'Chinese Spring' (CS) mutant *ph1b* (Sears' high pairing mutant; Sears, 1977) and durum wheat 'Cappelli' mutant *ph1c* (Giorgi's high pairing mutant; Giorgi, 1983). The latter was used to preferentially induce pairing between chromosomes of the D and S^{sh} genomes.

A *Triticum monococcum* subsp. *aegilopoides-Aegilops tauschii* amphiploid (genome A^mA^mDD) was obtained courtesy of Moshe Feldman (Weizmann Institute of Science, Rehovot, Israel). This amphiploid was prepared and analyzed by the late E.R. Sears and was found to be self-fertile (Sears 1941a, 1941b). Since this genotype does not possess the B genome and *Ph1* locus, it was used in the crossing procedure to preferentially induce pairing between chromosomes of the B and S^{sh} genomes.

An AG mutant, also in a CS genetic background, was obtained courtesy of Bernd Friebe (Kansas State University, Manhattan). It carries the EMS-mutated *Gc2* allele ($Gc2^{mut}$) on a homeologous distal translocation $4S^{sh}L$ of *Ae. sharonensis* in the wheat 4BL arm (T4BS×4BL-$4S^{sh}$L; Friebe et al., 2003). The transmission of gametes carrying these two alleles in heterozygous $Gc2^{mut}/Gc2$ plants is regular (random) rather than preferential, as occurs for the gametes with the *Gc2* allele.

The Israeli spring wheat elite cultivar Zahir (Hazera seed company, Israel) was used as the recurrent parent in this study. All of the abovementioned lines except AEG-1644 and AEG-2172 were susceptible to races TTKSK and PTKST.

Chromosome Doubling by Colchicine

To double the chromosomes of the wheat CS mutant *ph1b-AES* haploid hybrids, we used plants at the advanced tillering stage (Stage 2 in the scale of Zadoks et al., 1974). Plants were removed from their pots and washed thoroughly to remove any growth media particles. Then, the main tillers were separated out and immersed (to about 5 cm above the crown) in a colchicine solution (0.05% colchicine; crystalline; Sigma, 2% DMSO, 0.01% a.i. GA and 0.03% Tween 20) in tap water for 8 h. After this treatment, plants were washed, kept in running water overnight to remove residual colchicine, and replanted. Since the wheat-*AES* hybrid is haploid and self-sterile, the presence of a fertile sector in a spike indicated that the tissue and the seeds have a doubled chromosome number. Root tip counts of 56 chromosomes in the plant progeny verified their 8× ploidy level.

Mode of Selection for Resistance

Six plants from each recombinant line were grown at each generation. One spike of each plant was pollinated by Zahir, whereas the other spikes were allowed to self-pollinate (SP). Six to eight seeds from SP spikes of individual plants were shipped to either the University of Minnesota (St. Paul, MN, USA) or the University of the Free State (Bloemfontein, South Africa) for rust phenotyping against the pathogen races TTKSK or PTKST, respectively. The identification of resistant seedlings among the progenies of the SP spikes indicated that the mother plant

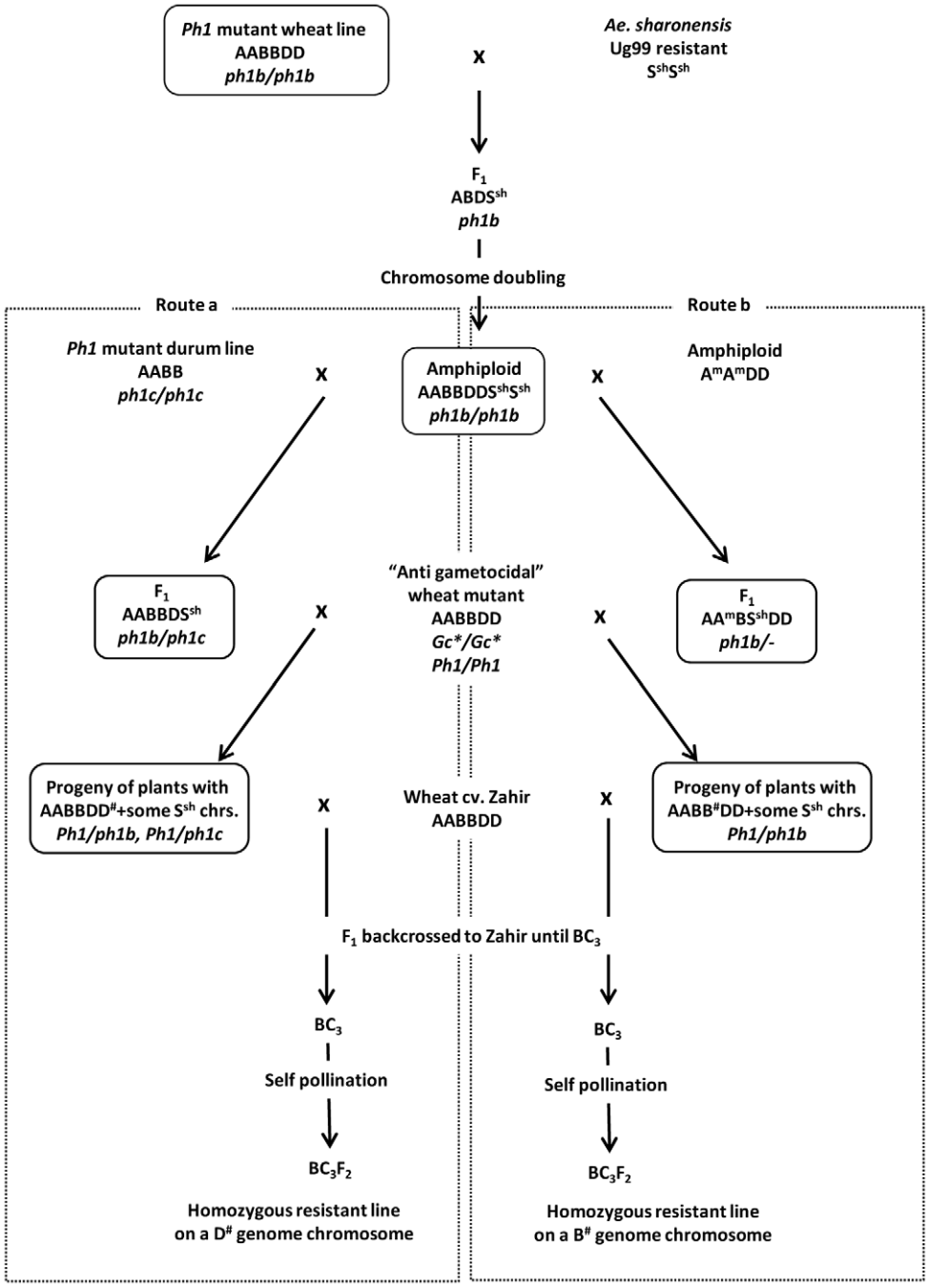

Fig. 1. Procedure for the production of wheat–*Aegilops sharonensis* recombinant lines resistant to stem rust. Route a and Route b describe production of D genome and B genome recombinants, respectively. Female parents are boxed. Superscript: # denotes recombinant chromosomes; * denotes mutant *Gc* allele.

was heterozygous for resistance and that seeds of resistant progenies are also expected from the crossed spike.

Procedure of the Introgression

The chromosome engineering procedure was designed to substitute a chromosome segment carrying the resistance from *AES* for its wheat homeologous segment in the desired genome, in this case B or D (Fig. 1). The genetic procedure was performed at the Institute for Cereal Crops Improvement, Tel Aviv University, Israel. The CS *ph1b* mutant was pollinated by the resistant *AES* accessions. Chromosomes of the hybrid were doubled using colchicine, and wheat-*AES* amphiploid seeds (AABBDDS^{sh}S^{sh}) homozygous for the *ph1b* mutation were obtained. The wheat-AEG-2172 amphiploids were pollinated by the Cappelli *ph1c* mutant, and seven seeds were obtained (Fig. 1, route a). Similarly, amphiploids of wheat-AEG-2172 or wheat-AEG-1644 were pollinated by the A^{m}A^{m}DD amphiploid, yielding six or three progeny, respectively (Fig. 1, route b).

These genotypes, possessing hemizygous genomes, were pollinated by the AG mutant. Each resistant plant derived from this cross was considered as a product of a

different homeologous recombination event, referred to here as a recombinant line. Yet some of these plants might have been derived from gametes carrying a nonrecombinant *AES* chromosome with the resistance gene. All these plants were crossed and backcrossed (BC) three times to the recurrent parent Zahir. At each generation, we selected plants based on desired morphology (short stature, large spike, plump seeds, etc.), low self-sterility, and expected resistance to race TTKSK as explained below.

Stem Rust Phenotyping

The rust reaction of seedlings was assayed against either *Pgt* race TTKSK or PTKST, both members of the Ug99 race group. Race PTKST occurs naturally in South Africa and allowed the convenience of year-round phenotyping. These two races have very similar virulence spectra based on the North American stem rust differential set with the exception that TTKSK is virulent for *Sr21* and avirulent for *Sr24*, whereas PTKST is avirulent for *Sr21* and virulent for *Sr24* (Singh et al., 2015).

Seedling resistance tests to race TTKSK (isolate: 04KEN156/04) were conducted in the Biosafety Level-3 (BSL-3) Containment Facility on the University of Minnesota, St. Paul campus, according to the protocols described by Scott et al. (2014). Evaluations to race PTKST (isolate: UVPgt60) were conducted at the University of the Free State in Bloemfontein, according to the protocols described in Pretorius et al. (2012).

Stem rust ITs, as described by Stakman et al. (1962), were assessed 12 to 14 d postinoculation based on uredinia development on susceptible controls. In the tests with race PTKST, wheat line Federation4*/Kavkaz carrying *Sr31* served as the susceptible check. The IT values of 2 or lower were considered incompatible (resistant), whereas ITs of 3 to 4 were considered compatible (susceptible).

Adult Plant Assessments in Rust Nurseries and Field Selections

One hundred and one lines at the BC_3F_2 or BC_2F_3 generations (backcrossed to Zahir), representing 16 recombination events, were space-planted in rows (20 to 30 seeds/row) in a stem rust nursery established at Greytown, South Africa, in 2013. Lines were selected based on the seedling response of a subsample to race PTKST. In addition to lines homozygous resistant or segregating, seven lines found homozygous susceptible at the seedling stage to PTKST were also included in the field trial. The stem rust susceptible line 37-07 (Prins et al., 2016) was included at 10-row intervals and as borders surrounding the entries in the field trial. The border rows of line 37-07 were inoculated with race PTKST to spread the inoculum onto the test entries. The recurrent parent Zahir was also included in the experiment. The severity of rust infection (from 0 to 100%) on the stems and leaf sheaths of plants was recorded based on the modified Cobb scale (Peterson et al., 1948). These severity values were accompanied by an infection response rating of R (resistant), MR (moderately resistant), MS (moderately susceptible), S (susceptible), or combinations thereof. Trace severities of stem rust infection were recorded as T.

Selections were made for highly resistant, self-fertile, and agronomically acceptable plant types (i.e., early heading and short statured, similar to Zahir) in Greytown, South Africa. Altogether, 154 plants were tagged for harvesting. Following progeny testing of these field selections with race PTKST at the seedling stage, 41 plants representing 10 recombination events were identified as homozygous resistant to PTKST. The 41 resistant selections and Zahir were field tested for a second season to race PTKST at Greytown in 2014 using the same methods described above.

Molecular Analysis of the Introgression Lines

High quality genomic DNA was obtained from the 41 resistant selections and their parental lines AEG-1644, AEG-2172, and Zahir using a standard CTAB method (Doyle and Doyle, 1990). The DNA was sent to the University of Bristol (Bristol, UK) for hybridization with the wheat 820K Axiom array. The Axiom Wheat HD Genotyping Array (Thermo Fisher Scientific, Inc., Waltham, MA) was used to genotype the 44 samples using the Affymetrix GeneTitan (Thermo Fisher Scientific, Inc.) system according to the procedure described by Affymetrix (Life Technologies, 2017). Allele calling was performed using the Affymetrix proprietary software package Affymetrix Analysis Suite, following the Axiom Best Practices Genotyping Workflow. A variant call rate threshold of 80% was used instead of the default value (97%) to account for the lower call rates typically obtained from hybridizing wheat relatives and progenitors to the array (Winfield et al., 2016). Assignment of a physical map position to the SNP markers was achieved by BLAST searching the probe sequences to the International Wheat Genome Sequencing Consortium (IWGSC) whole genome assembly v0.4 [available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies, verified 29 Sept. 2017]. The identification of putative introgressions was performed by comparing the genotype calls of the 41 recombinant hexaploid lines to their respective parental *Ae. sharonensis* accessions over a 10 SNP window and calculating a percentage match. Analysis of control introgression lines indicated that a match of 40% or higher within the 10 SNP window was indicative of an introgression in the Zahir background. This threshold was chosen based on the screening of known introgressions such as 1B/1RS. In addition, copy number variation (CNV) analysis was performed using the Affymetrix CNV Tool software. CEL files from the Axiom Wheat HD Genotyping Array were processed using Axiom Analysis Suite as described above. The annotation file was generated using the Affymetrix Annotation Converter. The CNV analyses were visualized in Biodiscovery Nexus Copy Number (BioDiscovery, El Segundo, CA; Supplementary Fig. S1).

Results

Production of Resistant Recombinant Lines and Molecular Characterization of Their A lien Introgressions

The crossing and selection procedure used in this study allowed for recombination between *AES* chromosomes carrying stem rust resistance and their wheat homeologues where most of the recurrent wheat genetic background was recovered. The large population that was obtained enabled selection of desired resistant wheat genotypes. In the end, we identified 32 homeologous recombinant lines derived from accession AEG-2172, of which 18 and 14 were the product of alien recombination targeted to chromosomes of the B or D genomes of wheat, respectively (Table 1). In nine other lines, recombination was targeted to occur between chromosomes of AEG-1644 and the B genome homoelogues of wheat.

Axiom wheat array analysis of the 41 homeologous recombinant lines and their parental lines revealed that large introgressions were present in all 41 lines (Fig. 2, Supplementary Fig. S1). The most consistent indication of recombination with *AES* was evident in chromosomes 1B, 1D, and 5B; yet sporadic large alien introgressions were also detected in chromosomes 1A (1 line), 2D (2 lines), 4D (1 line), 5D (2 lines), and 6A (2 lines; Fig. 2, Supplementary Fig. S1). In all these cases, alien chromatin was detected on a large part of the chromosome, ranging from 21 to 99% of its length. However, even when most of the wheat chromatin was substituted by that of *AES*, the telomeric region of the long arm of this chromosome was composed of wheat chromatin, indicating an interstitial introgression. Recombination event No. 36 in chromosome 1B was particularly noteworthy (Fig. 2) because about half of the chromosome, including the short arm, was substituted by *AES* chromatin. Additional smaller introgressions were also detected in chromosomes 1D, 3D, 4D, and 5B. In chromosome 1D, a small introgression was also detected in the parental wheat line, suggesting this is common to the hexaploid lines used in crossing. As expected, all of the lines that were derived from the same recombination event except one showed a similar SNP pattern. An unusual case was found with event No. 7: in line EM61/3, a recombination was detected in 5B, whereas in the remaining nine siblings, the recombinations were in 1D.

In most cases, alien introgression occurred in only one of the following chromosomes: 1B, 1D, or 5B. However, in the five EM107 lines of event No. 36, recombination was found in both 1B and 1D (Fig. 3).

In all of the lines derived from the five recombination events that were targeted to occur between chromosomes of AEG-2172 and those of the B genome, molecular data for recombination was found in either 5B or in 1B, as expected (in one case, EM5/1, in addition to 5B, smaller introgressions were detected also on 1B and 5D). Also, in two of three events targeted for the D genome alien introgression, recombination was found in 1D. However, when chromosomes of AEG-1644 were targeted to recombine with B genome chromosomes,

Table 1. Origin, seedling infection types (ITs), and field reactions of 41 selected recombinant lines and 'Zahir' wheat to infection by *Puccinia graminis* f. sp. *tritici* race PTKST.

Field selections 2013	Recombination event (genome)	Putative recombinant genome	*Ae. sharonensis* donor of resistance	Field scores†	PTKST IT range‡
EM5/1	2	B	AEG-2172	TR	;1
EM5/5	2	B	AEG-2172	TR	2-
EM11/4	5 (B)	B	AEG-2172	TMS	;1
EM15/5	5 (B)	B	AEG-2172	TR	;1
EM25/4	15 (B)	B	AEG-2172	TR	;1
EM26/3	15 (B)	B	AEG-2172	TR	;1
EM26/5	15 (B)	B	AEG-2172	TR	;1
EM30/1	19	B	AEG-2172	TR	;1=
EM30/2	19	B	AEG-2172	TR	;1=
EM30/3	19	B	AEG-2172	TR	; to ;1+
EM30/4	19	B	AEG-2172	TR	;1=
EM37/1	19	B	AEG-2172	TR	;1
EM37/2	19	B	AEG-2172	TR	;1
EM37/3	19	B	AEG-2172	TR	;1
EM37/4	19	B	AEG-2172	TR	;1
EM37/5	19	B	AEG-2172	TR	;1=
EM48/1	34	B	AEG-2172	TR-TS	;1
EM48/5	34	B	AEG-2172	TR-TS	;1=
EM54/1	5 (D)	D	AEG-2172	TR-TMS	;1= to X
EM56/1	5 (D)	D	AEG-2172	TR-30S	;1 to ;1++
EM56/5	5 (D)	D	AEG-2172	TR-30S	; to ;1+
EM61/3	7	D	AEG-2172	TR-10S	2
EM62/1	7	D	AEG-2172	TR-60S	;1=
EM62/4	7	D	AEG-2172	TR-60S	;1=
EM63/1	7	D	AEG-2172	TR	;1+
EM63/2	7	D	AEG-2172	TR	;1
EM63/3	7	D	AEG-2172	TR	;1
EM63/4	7	D	AEG-2172	TR	;1++ (X)
EM63/5	7	D	AEG-2172	TR	;1 to ;12
EM71/2	7	D	AEG-2172	TR-5S	;1
EM71/4	7	D	AEG-2172	TR-5S	;1=
EM75/5	15 (D)	D	AEG-2172	TR-5S	11+
EM83/2	1	B	AEG-1644	TR	; to ;1+
EM86/2	1	B	AEG-1644	TR-20S	;
EM86/4	1	B	AEG-1644	TR-20S	0;
EM97/2	36	B	AEG-1644	TR-5S	0;
EM107/1	36	B	AEG-1644	TR	;1=
EM107/2	36	B	AEG-1644	TR	;1=
EM107/3	36	B	AEG-1644	TR	;1=
EM107/4	36	B	AEG-1644	TR	;1=
EM107/5	36	B	AEG-1644	TR	;1=
Zahir				40MS	4

† Stem rust ratings (0–100%) according to modified Cobb scale. Response types are indicated by R (resistant), MR (moderately resistant), MS (moderately susceptible), and S (susceptible). T represents trace severity of rust infection.

‡ Seedling ITs (0–4 scale) of progenies of plants selected as resistant in the field.

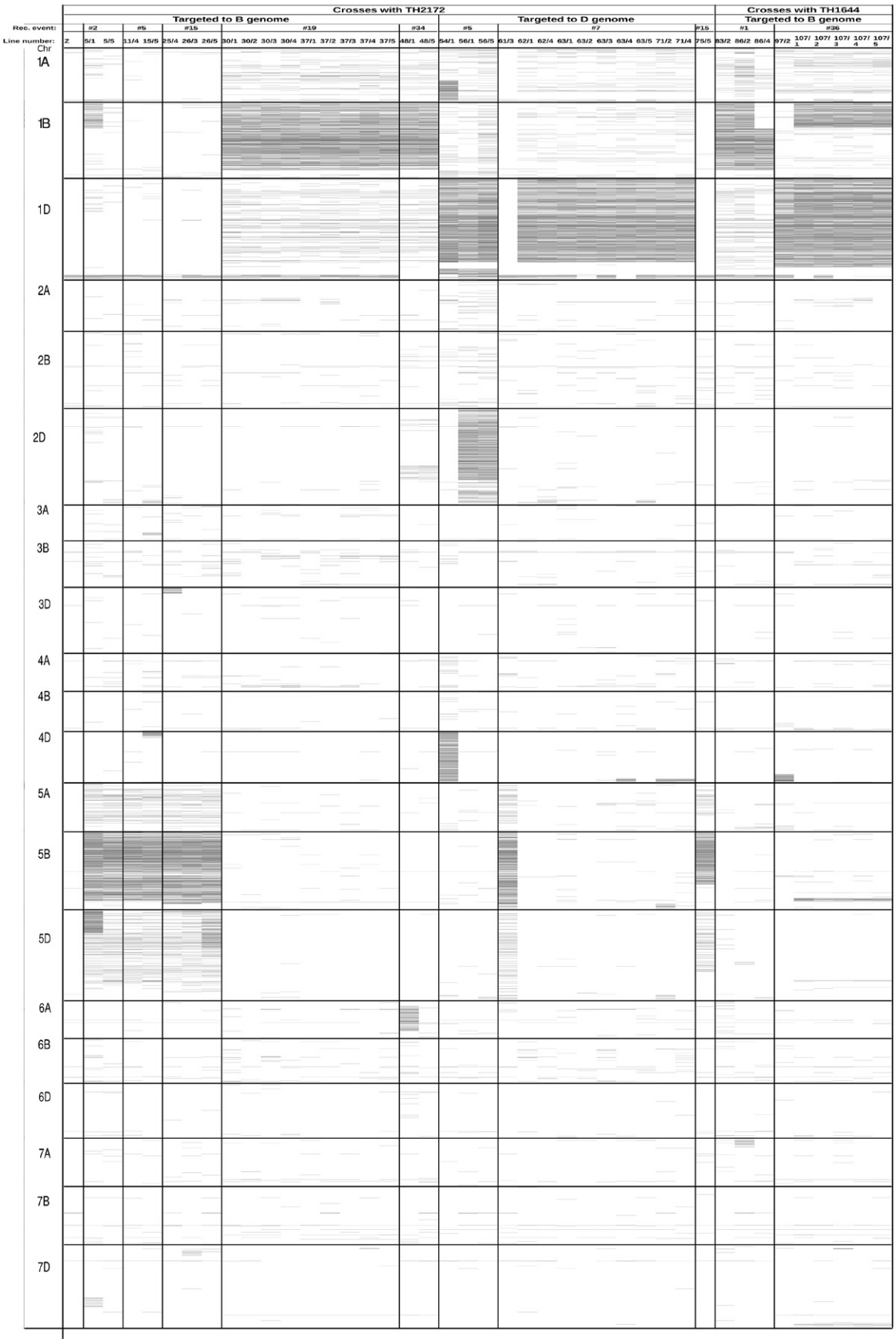

Fig. 2. Axiom wheat array analysis of 41 recombinant lines resistant to African *Puccinia graminis* f. sp. *tritici* races in the Ug99 lineage. Only chromosomes with consistent *AES* SNP (red color) are shown. Genotype calls assigned in the spreadsheet reflect the relative match to *AES* over a 10 SNP window. A score over 40% is considered indicative of introgressed material and is highlighted. Score position on the chromosome is shown from short arm telomere (top) to long arm telomere (bottom).

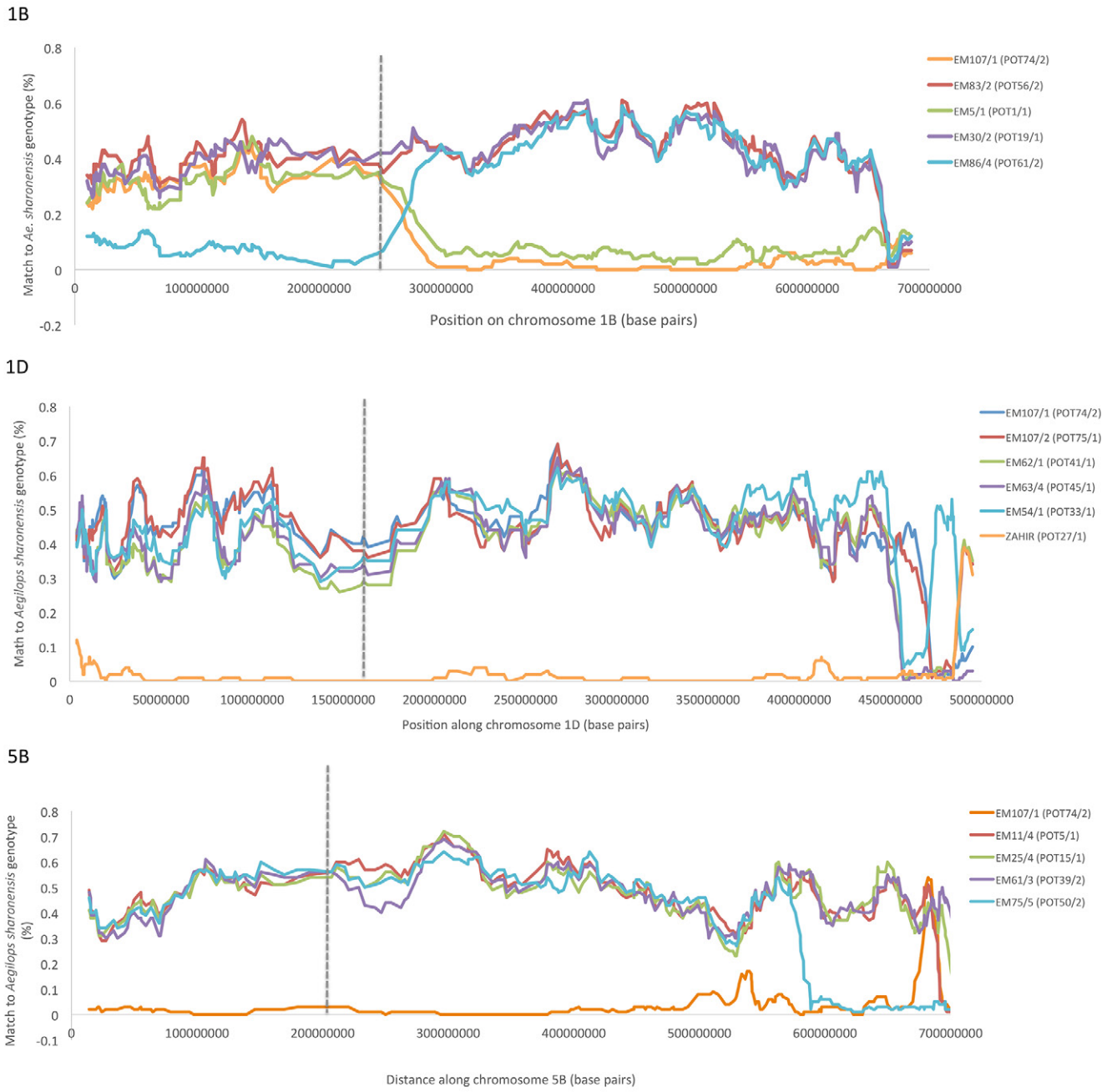

Fig. 3. Percentage match of recombinant lines to their parental *Ae. sharonensis* accession for recombinant chromosome 1B, 1D, or 5B. Vertical dashed lines denote centromere position.

molecular evidence for alien chromatin was detected in either 1B (one event) or in both 1B and 1D (one event).

Seedling Reaction of Selected Lines to *Pgt* Races TKTTF and TRTTF

Twelve of the 41 PTKST-resistant lines were also evaluated against TKTTF (06YEM34-1) and TRTTF (13ETH18-1) in the BSL-3 facility in Minnesota to determine if they carry resistance to widely virulent *Pgt* races outside the Ug99 race group. Race TKTTF was responsible for the recent epidemic (2013–2014) on wheat cultivar Digalu (carrying *SrTmp*) in Ethiopia and differs from race TTKSK with respect to virulence for *Sr36*, *Sr17*, and *SrTmp* and avirulence for *Sr11* and *Sr31* (Olivera et al., 2012, 2015). Race TRTTF was first identified in Yemen in 2006, but has subsequently been reported in Ethiopia. It differs from race TTKSK with respect to virulence for *Sr36* and *SrTmp* and avirulence for *Sr8a* and *Sr31* (Olivera et al., 2012).

From the replicated seedling tests of these 12 lines, three were found resistant (IT = ;1–) to both TKTTF and TRTTF, and the rest were susceptible. All of the resistant lines were derived from recombination event No. 36 with accession AEG-1644.

Phenotyping Lines in the Field

Resistant, susceptible, and segregating lines were identified in the 2013 field nursery (Fig. 4). The response of individual plants within resistant lines ranged from TR- to TMRMS-TMS and those for segregating lines ranged from TR-TS to TR-60S (Table 1). The response of individual plants within susceptible lines ranged from TS-20S to 30MS-40S. Between-row variation of susceptible lines varied from TS to 40S with Zahir scoring at 40MS and Line 37-07 at 60S to 80S. One-hundred and fifty-four single plants showing high levels of stem rust resistance were selected, harvested, and tested for homozygosity of resistance. Of these 154 tested plants, 41 yielded homozygous resistant BC_3F_3 progenies (some of BC_2F_3 generation), some with high levels of resistance to race PTKST (Fig. 4). The ITs of resistant progeny ranged from 0; to 2–. Although the 41 selections were all resistant in the progeny tests, the ITs in seven lines were not uniform. For example, within-line ITs varied from ; to ;1+ and ;1 to ;1++. Zahir exhibited a fully susceptible IT 4 to PTKST at the seedling stage.

In the confirmatory field evaluation in 2014, all 41 lines were resistant to race PTKST. Most lines exhibited reactions of TR, but ranged from 0R to 5MS (Supplementary Table S1). Within-line variation was observed in five entries (e.g., 0R-TMR, 0R-10MR, and 0R-15MRMS). Although these lines were not uniform in their response, the phenotypes were nevertheless all in the resistant category. Line 37-07 and Zahir were scored as 80S and 40S, respectively. Five entries showed hybrid necrosis, resulting in the premature death of foliage. Other lines segregated for plant height or the presence of awns, and several lines were susceptible to the prevailing leaf rust (*Puccinia triticina*) race at Greytown. In a minority of the selections, conspicuous stem discoloration was observed, a distinct phenotype typically seen in wheat lines carrying the stem rust resistance gene *Sr2*.

Discussion

The genetic procedure described in this investigation was designed to allow for the controlled transfer of resistance gene(s) into the desired B or D genome of bread wheat. Accordingly, by crossing a wheat-*AES* amphiploid with a tetraploid genotype deficient for one of the bread wheat genomes (either B or D), we markedly increased the probability of pairing and recombinations between chromosomes of *AES* and the hemizygous wheat genome. Indeed, Sears (1977) previously demonstrated that homologous pairing is preferential over homeologous pairing, even in a homozygous *ph1b* mutant, and also that *ph1b* (lacking *Ph1*) promotes homeologous pairing between chromosomes of hemizygous (haploid) genomes. The chromosome engineering method that was developed in this research was efficient in producing a high number of recombinant lines, from which those having stem rust resistance, superior agronomic traits, and good fertility could be selected (Table 1). In previous work (Millet et al., 2014), a simpler method of pairing induction in a haploid hybrid yielded only a small number of recombinant lines due to low fertility of the hybrid.

Homeologous pairing is expected to occur between all chromosomes of the hemizygous genomes, but backcrossing to a recurrent wheat parent with subsequent selection for the target trait will maintain only the recombinant chromosomes carrying the trait under selection. Moreover, the backcrossing procedure may eliminate nonrecombinant monosomic alien addition progeny with resistance since the alien chromosome will be transmitted only in a low rate to the next generation.

Considering the early generation of the lines (BC_2 and BC_3), sporadic introgressions are also expected, but are considered as not related to the resistance phenotype and likely will diminish in further generations of backcrossing and selection for *Pgt* resistance.

Genotyping the recombinant lines with the ultra-high-density Axiom array was found to be an efficient tool for detecting the boundaries of alien chromatin introgressed into specific chromosomes of the wheat genome.

Provided that homeologous pairing was induced, one would expect that the recombined wheat chromosome as reflected in the Axiom array data (Fig. 2) indicates its homeologue of the diploid *AES* resistance donor, AEG-1644 or AEG-2172. Indeed, Yu et al. (2017) found that AEG-1644 carries a TTKSK resistance gene of major effect on $1S^{sh}$ and one with minor effect on $5S^{sh}$. This was confirmed in our work when recombination was consistently found in all 41 selected resistant lines, on wheat chromosome 1B, 1D, or 5B, and particularly in that all the AEG-1644 recombinant lines possessed large introgressions in chromosomes 1B and 1D (Fig. 2, Supplementary Fig. S1). In all of these chromosomes, wheat chromatin was detected on the long arm telomere, which indicates that they are recombinant chromosomes. EM86/4 was the only resistant line possessing alien chromatin in the long arm but not in the short arm of 1B or in any other chromosome (Fig. 2 and 3). This result suggests that the gene conferring resistance to Ug99 group races is located on chromosome 1B, between 280 and 650 Mbp (Fig. 3).

Yu et al. (2017) also suggested that chromosome $5S^{sh}$ of AEG-1644 carries a major effect resistance gene against race TRTTF and a minor effect gene against the race on $1S^{sh}$. Whether the three lines that were tested and found resistant to race TRTTF in this study acquired their resistance from $1S^{sh}$ or from $5S^{sh}$ is still unclear. Chromosome 5B of these lines carries a very small introgression at the 5BL telomeric region, whereas chromosomes 1B or 1D carry larger introgressions—but, according to Yu et al. (2017), the effect of the gene on $5S^{sh}$ is stronger than that on $1S^{sh}$. However, mapping data for the latter suggest that the gene is most likely located in the middle (~48 cM) of chromosome $1S^{sh}$, thus precluding it from lying in the 5BL telomeric region. The differences observed for the phenotypic effect of the genes may be due to inhibitors that are present in the donor parent, but not in wheat.

Since the progenies from the cross of wheat AEG-2172 with the *ph1c* durum mutant have the genomic

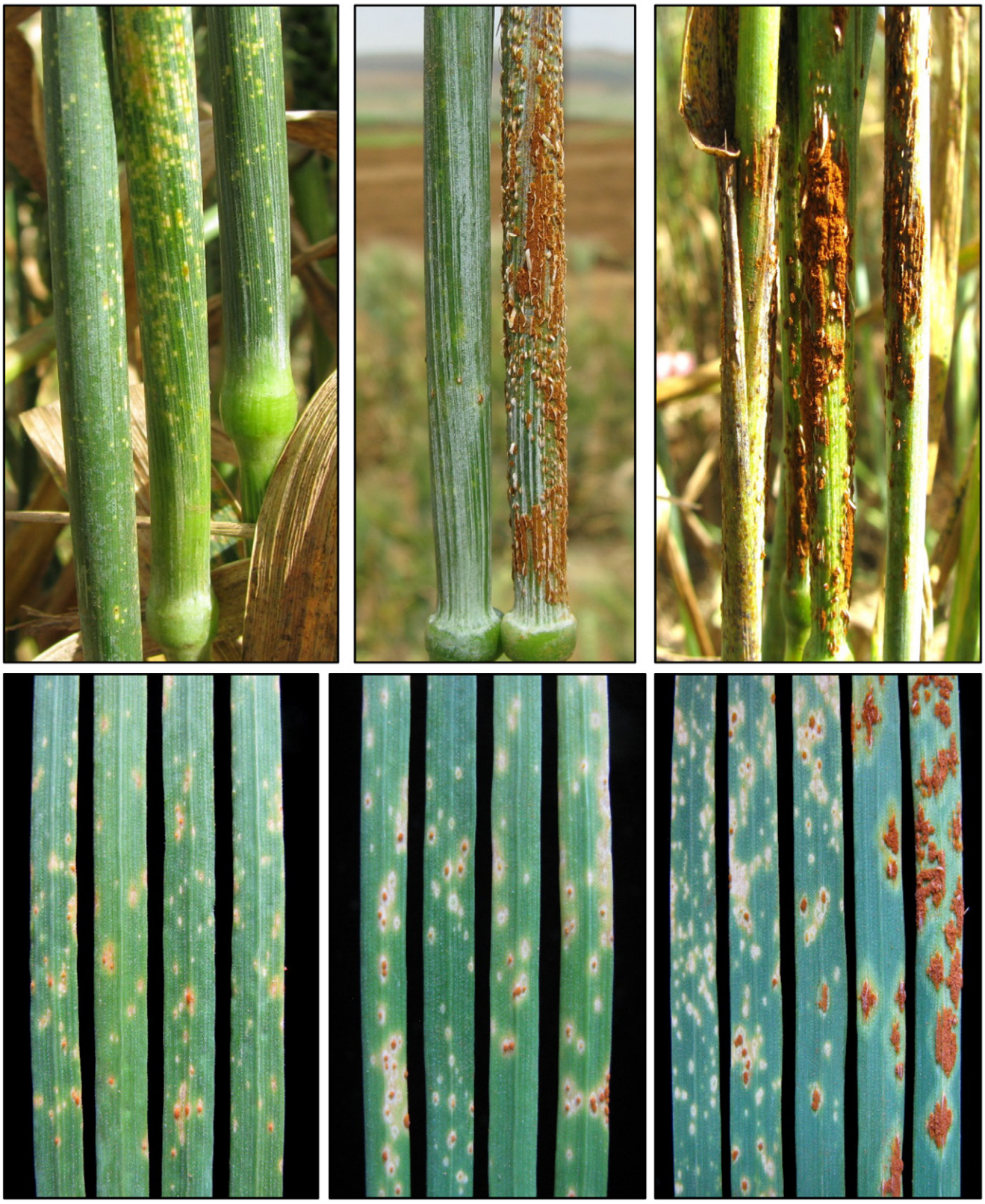

Fig. 4. Top: Stem rust response of different lines in the field nursery at Greytown in 2013; (left to right) lines EM30 (resistant), EM62 (segregating), and EM10 (susceptible). Bottom: seedling infection types obtained in progeny tests of single plants selected as resistant in the field; (left to right) lines EM62-1 (homozygous resistant), EM48-5 (homozygous resistant), and EM53-2 (segregating).

constitution of AABBDS^{sh} (i.e., chromosomes of only the D and S^{sh} genomes are hemizygous and the rest are homozygous), it is expected that most pairing and recombination events will take place between chromosomes of the D and S^{sh} genomes. This was the case for most of the recombinant lines based on the Axiom array data. However, in two cases (EM61/3 and EM75/5), recombination occurred unexpectedly in 5B instead of 5D.

Similarly, when amphiploids of wheat and either AEG-2172 or AEG-1644 were pollinated by the A^{m}A^{m}DD amphiploid, progenies hemizygous for the B and S^{sh} genomes were obtained. Although genomes A of wheat and A^{m} of *T. monococcum* subsp. *aegilopoides* are not true homologs, they are still compatible and produce regularly at meiosis about five bivalents (Johnson and Dhaliwal, 1976). Hence, homeologous pairing in the absence of *Ph1* is expected to occur mainly between the B and S^{sh} genomes.

Indeed, the results from the Axiom array indicated that in all five recombination events involving the cross with AEG-2172 and targeted to the B genome, recombination occurred between chromosomes 1B or 5B and their S^{sh} genome homeologues only. Three events produced a recombined 5B chromosome, and two events produced a recombined 1B chromosome. However, when AEG-1644 was used in the cross, one event produced a recombinant 1B chromosome and the other produced recombinant 1B and 1D chromosomes. This latter result was unexpected since it theoretically indicates recombination of the alien 1S^{sh} chromosome with both the 1B and 1D wheat chromosomes—a rare event that is possible in a 1B–1S^{sh}–1D trivalent, but nevertheless not expected to yield similar alien translocations on the short arms of 1B and 1D.

To avoid a possible gametocidal effect (e.g., Endo 1990), genotypes with hemizygous genomes were pollinated by the AG mutant. Each seed that was obtained from this step reflects a single recombination event between wheat and the *AES* chromosomes with the possibility that the recombination includes the target resistance gene(s). From the crosses with the AG mutant, 16, 51, and 77 seeds were obtained for the combinations of AEG-2172 with Capelli *ph1c* mutant, AEG-2172 with A^{m}A^{m}DD amphiploid, and AEG-1644 with A^{m}A^{m}DD amphiploid, respectively. The resulting progenies were heterozygous *Gc2/Gc2^{mut}*, allowing for normal segregation of these genes in further generations and for selection against the *Gc2* allele by discarding individual plants showing around 50% female sterility as expressed by seed set in SP spikes of hemizygous *Gc2* plants.

To recover the genetic background of bread wheat, we performed a cross and three BCs to the recurrent parent Zahir. This was accompanied by morphological selection for desired agronomic traits and seed set (to select against the gametocidal gene *Gc2*) and indirect selection for rust resistance by analysis of the SP siblings. Direct phenotypic selection for resistance was not possible since the Ug99 race group has not yet been found in Israel, but the resistance was finally confirmed in South Africa or in Minnesota. As a result of this selection, 41 resistant BC_3F_2 (including some BC_2F_3) lines remained, representing three recombination events between chromosomes of the D genome and S^{sh} of AEG-2172, five between B and S^{sh} of AEG-2172, and two between B and S^{sh} of AEG-1644.

Conclusions

We demonstrated that, in most cases, it was possible to target *AES* introgressions carrying stem rust resistance genes into a selected wheat genome. *AES* chromatin contributed the most effective resistance genes against widely virulent *Pgt* races (including the Ug99 race group as well as TRTTF and TKTTF) as expressed by ITs at the seedling stage and severity/reaction scores at the adult plant stage in the field of derived wheat introgression lines (Table 1, Fig. 4). Both *AES* parental lines AEG-2172 and AEG-1644 conferred a similarly high level of resistance in their derived progenies. The fact that not all lines resistant to race PTKST were equally resistant to races TRTTF or TKTTF suggests that the gene(s) transferred from *AES* is race-specific and should not be deployed singly.

Backcrossing of the introgression lines is being continued to reconstitute the recurrent wheat parent of Zahir. This should recover more of the wheat genetic background and make lines more suitable for accurate mapping of the alien segment and for rigorous field evaluations. Such field evaluations will provide valuable data as to whether there might be a yield penalty associated with linkage drag, and may show which of the targeted B or D wheat genome chromosomes are preferred in recombination.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest.

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Supplemental Information Available

Supplemental information is included with this article.

Supplementary Fig. S1. Copy number variation (CNV) plots in the genome of all recombinant (EM) lines and Zahir. Consistent lower values indicate alien chromatin.

Supplementary Table S1. Field response of 41 selected wheat–*Ae. sharonensis* introgression lines to race PTKST at Greytown, South Africa, in 2014. Entries were confirmed as homozygous for resistance to stem rust in seedling tests.

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